U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NO.: 202531

U.S. APPLICATION NO.: 09/402721

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US98/06969	07 APRIL 1998 (07.04.98)	08 APRIL 1997 (08.04.97)

TITLE OF INVENTION
METHOD FOR PRODUCING BEER

APPLICANT(S) FOR DO/EO/US
PELZ, Dieter; MOSER, Gilbert; ZANKER, Gerald; SERRO, Walter; RIBITSCH, Volker; RANDHAHN, Horst; DEGEN, Peter J.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) now rather than delay examination until the expiration of the applicable time limit set forth in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendment to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☒ A copy or translation of the Amendments made by the Applicant during PCT Chapter II, which are attached as Annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 17 below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment. ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☐ A verified small entity statement.

17. ☐ Other items or information:

U.S. APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/402721	PCT/US98/06969	202531

18. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO ..... $ 840.00	$840.00	
International preliminary examination fee paid to USPTO ..... $ 670.00		
No international preliminary examination fee paid to USPTO, but international search fee paid to USPTO ..... $ 760.00		
Neither international preliminary examination fee nor international search fee paid to USPTO ..... $ 970.00		
International preliminary examination fee paid to USPTO and all claims satisfied provisions of PCT Article 33(1) to (4) ..... $ 96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT=	$840.00	
Surcharge of $130.00 for furnishing the National fee or oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date		

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total Claims	39	-20=	19	x $ 18.00	$342.00	
Independent Claims	3	- 3 =	0	x $ 78.00	$0.00	
☐ Multiple Dependent Claim(s) (if applicable)				+$260.00	$	
				TOTAL OF ABOVE CALCULATIONS=	$1,182.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed.					$	
				SUBTOTAL=	$	
Processing fee of **$130.00** for furnishing English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date.					$	
				TOTAL NATIONAL FEE=	$	
Fee for recording the enclosed assignment. The assignment must be accompanied by an appropriate cover sheet. **$40.00** per property +					$	
				TOTAL FEE ENCLOSED=	$1,182.00	
				Amount to be: refunded	$	
				charged:	$	

a. ☒ A check in the amount of $1,182.00 to cover the above fee is enclosed.

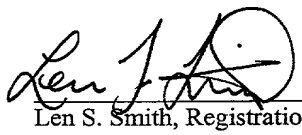
b. ☐ Please charge Deposit Account No. 12-1216 in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 12-1216. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

LEYDIG, VOIT & MAYER, LTD.
Two Prudential Plaza, Suite 4900
180 North Stetson
Chicago, Illinois 60601-6780
(312) 616-5600 (telephone)
(312) 616-5700 (facsimile)



Len S. Smith, Registration No. 43,139
One of the Attorneys for Applicant(s)

U.S. APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/402721	PCT/US98/06969	202531

CERTIFICATION UNDER 37 C.F.R. § 1.10

"Express Mail" Label Number: EL305738184US

Date of Deposit: October 8, 1999

I hereby certify that this express request to begin national examination procedures under 35 U.S.C. § 371(f) of the International Patent Application referenced above, including all of the items listed thereon as enclosures, is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to Box PCT, Assistant Commissioner for Patents, Attention: DO/EO/US, Washington, D.C. 20231.

Irina L. Mikitiouk
Printed Name of Person Signing

I. Mikitiouk
Signature

PATENT
Attorney Docket No. 202531

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Pelz et al.

Group Art Unit: Unassigned

U.S. National Phase of:
PCT/US98/06969

Examiner: Unassigned

International Filing Date:
April 7, 1998

For: METHOD FOR PRODUCING BEER

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments and consider the following remarks.

AMENDMENTS

IN THE CLAIMS:

Please cancel claims 6, 19, and 23.

Please amend the remaining pending claims as follows:

1. (Amended) A method for producing beer comprising:

(a) filtering beer through a porous membrane until such time that the [said] porous membrane is in need of cleaning,

(b) contacting the [said] porous membrane with an enzyme selected from the group consisting of cellulases, amylases, and combinations thereof in the absence of a protease or a glucanase to clean the [said] porous membrane, and

(c) then reusing the [said] porous membrane to continue filtering beer.

2. (Amended) The method of claim 1, wherein the [said] porous membrane is not contacted with an enzyme other than the [said] cellulase or the [said] amylase.

3. (Amended) The method of claim 1 [or 2], wherein the [said] porous membrane is contacted with the [said] cellulase.

4. (Amended) A method for producing beer comprising:

(a) filtering beer through a porous membrane until such time that said porous membrane is in need of cleaning,

(b) contacting the [said] porous membrane with a cellulase having a crystalline:soluble cellulase activity ratio at 60 minutes of at least about 0.1 to clean the [said] porous membrane, and

(c) then reusing the [said] porous membrane to continue filtering beer.

5. (Amended) The method of claim 4 [3 or 4], wherein the [said] porous membrane is contacted with the [said] cellulase and is not contacted with any other enzyme.

7. (Amended) The method of claim 4 [6], wherein the [said] cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.3.

8. (Amended) The method of claim 7, wherein the [said] cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.4.

9. (Amended) The method of claim 8, wherein the [said] cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.5.

10. (Amended) The method of claim 9, wherein the [said] cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 1.

11. (Amended) The method of claim 10, wherein the [said] cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 1.2.

12. (Amended) The method of claim 4 [any of claims 1-11], wherein the [said] cellulase is derived from *Trichoderma*.

13. (Amended) The method of claim 12, wherein the [said] *Trichoderma* is *Trichoderma reesei* or *Trichoderma longibrachiatum*.

14. (Amended) The method of claim 4 [any of claims 1-11], wherein the [said] cellulase is derived from *Thermomonospora*.

15. (Amended) The method of claim 14, wherein the [said] *Thermomonospora* is *Thermomonospora fusca*.

16. (Amended) The method of claim 4 [any of claims 1-3 and 6-15], wherein the [said] porous membrane is contacted with an [said] amylase.

17. (Amended) The method of claim 16, wherein the [said] amylase is selected from the group consisting of α-amylase, β-amylase, and the combination thereof.

18. (Amended) The method of claim 4 [any of claims 1-17], wherein the method further comprises contacting the [said] porous membrane [is additionally contacted] with an aqueous base prior to reusing the [said] porous membrane [being reused].

20. (Amended) The method of claim 18 [or 19], wherein the [said] aqueous base is an aqueous solution of NaOH and/or KOH.

21. (Amended) The method of claim 18 [any of claims 18-20], wherein the [said] base is present in a concentration of 0.1-1 N in the [said] aqueous base.

22. (Amended) The method of claim 18 [any of claims 1-21], wherein the [said] porous membrane is contacted with the [said] aqueous base at a temperature of 40-90 °C.

24. (Amended) The method of claim 4 [any of claims 1-3 and 6-23], wherein the [said] porous membrane is contacted with α-amylase at a temperature of 60-75 °C and a pH of 4.6-5.8.

25. (Amended) The method of claim 4 [any of claims 1-3 and 6-23], wherein the [said] porous membrane is contacted with β-amylase at a temperature of 40-60 °C and a pH of 4.6-5.8.

26. (Amended) The method of claim 4 [any of claims 1-25], wherein the [said] porous membrane is cleaned until the zeta potential of the [said] porous membrane ceases to change.

27. (Amended) The method of claim 4 [any of claims 1-26], wherein the [said] time that the [said] porous membrane is in need of cleaning is determined by the pressure drop across the [said] porous membrane.

28. (Amended) The method of claim 4 [any of claims 1-26], wherein the method further comprises determining the [said] time that the [said] porous membrane is in need of cleaning [is determined] by determining the streaming potential or zeta potential of the [said] porous membrane.

29. (Amended) A method for producing beer comprising:

(a) filtering beer through a porous membrane that progressively clogs during filtration,

(b) monitoring the streaming potential or zeta potential of the [said] porous membrane as a measure of the extent of clogging of the [said] porous membrane,

(c) halting filtration of the beer through the [said] porous membrane before the [said] porous membrane becomes fully clogged as determined by the streaming potential or zeta potential of the [said] porous membrane,

(d) cleaning the [said] porous membrane, and

(e) then reusing the [said] porous membrane to continue filtering beer.

30. (Amended) The method of claim 28 [or 29], wherein the [said] filtration is halted when the streaming potential or zeta potential of the [said] porous membrane is reduced to 20% of its original value for the unused porous membrane.

31. (Amended) The method of claim 4 [any of claims 1-30], wherein the [said] porous membrane is a polyamide porous membrane.

32. (Amended) The method of claim 31, wherein the [said] filtration is halted when the zeta potential of the [said] porous membrane exceeds -5 mV as measured at pH 4.2.

33. (Amended) The method of claim 4 [any of claims 1-32], wherein the [said] filtering of the beer is cold-filtering of the beer.

34. (Amended) A filtration unit for filtering beer comprising a feeder line for the filtration-bound beer, a porous membrane, a run-off line for the filtered beer, and means for monitoring the streaming potential and/or zeta potential of the [said] porous membrane through which beer flows.

35. (Amended) The filtration unit of claim 34, further comprising a bypass porous membrane through which beer flows, wherein the [said] monitoring means for monitoring the streaming potential and/or zeta potential does so with respect to the [said] bypass porous membrane.

In addition, please add the following new claims:

36. The method of claim 29, wherein performing step (d) of the method comprises contacting the porous membrane with a cellulase having a crystalline:soluble cellulase activity ratio at 60 minutes of at least about 0.1 to clean the porous membrane.

37. The method of claim 4, wherein the porous membrane is a nylon-6,6 membrane.

38. The method of claim 4, wherein the porous membrane has a pore rating of about 0.02-1 μm.

39. The method of claim 38, wherein the porous membrane has a pore rating of about 0.1-1 μm

40. The method of claim 39 wherein the porous membrane has a pore rating of about 0.45 μm.

41. The method of claim 4, wherein the method further comprises pre-filtering the beer before performing step (a) of the method.

42. The method of claim 41, wherein the beer is pre-filtered through Diatomateous earth or a combination of Diatomteous earth and deep-bed filtration.

REMARKS

The present application is the U.S. national phase of a PCT application. The claims and specification of the PCT application were amended during the Chapter 2 phase before the International Patent Examining Authority. Specifically, substitute pages 9, 11-13, 15, 16, 18, and 29, which revised the specification and claims, were submitted. In the present Amendment, originally filed claims 6, 19, and 23 are canceled, and claims 36-42 are added. For the Examiner's convenience, applicants have attached a copy of the pending claims (as amended) hereto.

The pending claims which are not cancelled by this Amendment have been amended merely to conform the claims to U.S. patent practice (e.g., by eliminating multiple dependent claims which were dependent on other multiple dependent claims) or to more clearly and particularly describe the invention (e.g., replacement of "said" with "the" elimination of passive voice, etc.). The cancellation of claims 6, 19, and 23 is likewise intended merely to avoid redundancy in the previously filed claims or to organize previously multiply dependent claims in a more coherent fashion. Thus, the present amendment of the claims should in no way be regarded as a surrender of subject matter or scope of the claimed invention, or as otherwise limiting the claims set forth herein.

The addition of new claims 36-42 is supported by the specification in its entirety and claims 1-35 as originally filed. Examples of support for claim 36 can be found on page

5, lines 11-17 and 21-27,as well as page 6, lines 4-12 of the specification. Examples of support for claims 37-40 can be found at page 6, lines 13-30. Examples of support for claims 41 and 42 can be found at page 9, lines 8-13.

In view of the above, the amended claims and the newly added claims are supported by the specification and do not represent new matter. Furthermore, the application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the instant application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

Len S. Smith, Reg. No. 43,139
One of the Attorneys for Applicants
LEYDIG, VOIT & MAYER, LTD.
Two Prudential Plaza, Suite 4900
180 North Stetson
Chicago, Illinois 60601-6780
(312) 616-5600 (telephone)
(312) 616-5700 (facsimile)

Date: October 8, 1999

M:\Clients\Pall\Amd\202531prelim-amdt.doc

METHOD FOR PRODUCING BEER

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of producing beer, particularly of filtering beer through a filtration medium and cleaning the filtration medium with enzymes such that it can be reused in beer filtration.

BACKGROUND OF THE INVENTION

In view of the extended marketing channels, germs (e.g., bacteria) have to be removed from the beer in order to make it storable. Nowadays, germ removal is mainly carried out by pasteurization of the beer. To this end, the beer is, for example, bottled or canned, and heated to a temperature of between 62 and 69 °C to kill the germs.

This pasteurization does, however, involve considerable energy consumption. It has the further disadvantage that the energy introduced can trigger chemical reactions which impair the product and are difficult to control. These reactions can, for example, adversely affect the flavor of the product ("pasteurized taste"), and there is also the danger that undesired substances will form. Pasteurization is, therefore, a relatively expensive germ removing method involving high energy expenditure and, consequently, having harmful effects on the environment as well as reducing the quality of the product.

Another known germ removing method is cold-filtration. Cold-filtered beer is available as so-called "draft beer" in, for example, the United States, Japan and Korea. This beer is prohibited in Europe because it contains technical enzymes.

These technical enzymes are present in the beer to counteract a drawback inherent in the cold-filtration method: early clogging of the filter. This clogging is due to deposits of substances to be filtered out of the

beer on the upstream side of the filter, e.g., a membrane filter. The deposits are difficult or even impossible to remove from the filter and reduce the service life of the filter. This increases the cost of producing the beer as membrane filters are expensive.

To prolong the service life of the filter, the manufacturers of membrane filters recommend cleaning the used membranes by treating them with proteases, glucanases, and xylanases, as well as with chemicals such as surfactants, acids/bases, and oxidizing agents, to make them reusable. This cleaning can be carried out at, for example, two stages, with the above-mentioned enzymes at a first stage, followed optionally by additional cleaning with the above-mentioned chemicals in a second stage.

The literature also discloses methods of cleaning membrane filters used in filtering beer, which cleaning methods involve a variety of techniques. For example, U.S. Patent 5,227,819 discloses a method for the cleaning of a polyamide microporous membrane used in cold-filtering beer by passing a dilute alkaline solution through the microporous membrane. International Patent Application WO 96/23579 discloses a somewhat different method of cleaning a membrane filter used in beer filtration. That method is characterized by treating the membrane filter with an enzyme-containing aqueous solution of β-glucanases, xylanases, and cellulases, cleaning the membrane filter with an acidic aqueous cleaning solution, and cleaning the membrane filter with a peroxide-containing alkaline cleaning solution.

Given, for example, a filter area of approximately 320 m^2, a cleaning procedure will, by way of example, make provision for enzymatic cleaning after every 5,000 hectoliters filtered and an additional chemical cleaning after every 20,000 hectoliters filtered. The typical service life of filters with the above-mentioned filter area of approximately 320 m^2 having undergone the

manufacturer-recommended cleaning is approximately 100,000 hectoliters.

The previously known cleaning procedures do, however, have the disadvantage that they are unable to remove the deposits on the filter to a satisfactory extent, which causes the cleaning efficiency to diminish strongly as the membrane filter increases in age.

Yet another disadvantage is the sudden, random clogging of the filter membrane, unrelated to standard norms like total nitrogen content, or percent of original wort. A fully clogged membrane filter cannot be satisfactorily cleaned under procedures following the current state of technology, which greatly reduces the service life of the filter. It is difficult to determine when a filter will become so clogged that it cannot be satisfactorily cleaned, and, therefore, a filter may be cleaned prematurely or not in time, i.e., too early or too late.

In view of the foregoing problems, there exists a need for an improved method of producing beer, particularly wherein the beer can be filtered through a filtration medium that can be satisfactorily cleaned and reused. The present invention provides such a method. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a method for producing beer comprising filtering beer through a porous membrane until such time that the porous membrane is in need of cleaning, contacting the porous membrane with an enzyme selected from the group consisting of cellulases, amylases, and combinations thereof, particularly a cellulase having a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.1, to clean the porous membrane, and then reusing the porous membrane to

continue filtering beer. The present invention further provides a method for producing beer comprising filtering beer through a porous membrane that progressively clogs during filtration, monitoring the streaming or zeta potential of the porous membrane as a measure of the extent of clogging of the porous membrane, halting filtration of the beer through the porous membrane before the porous membrane becomes fully clogged as determined by the streaming or zeta potential of the porous membrane, cleaning the porous membrane, and then reusing the porous membrane to continue filtering beer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of beer filtration amount (g) versus filtration time (sec) in connection with filtering beer through a previously unused, i.e., a new, porous membrane.

Figure 2 is a graph of beer filtration amount (g) versus filtration time (sec) in connection with filtering beer through a clogged porous membrane.

Figure 3 is a graph of beer filtration amount (g) versus filtration time (sec) in connection with filtering beer through a previously clogged porous membrane cleaned in accordance with a prior art technique.

Figure 4 is a graph of beer filtration amount (g) versus filtration time (sec) in connection with filtering beer through a previously clogged porous membrane cleaned in accordance with the present invention.

Figure 5 is a schematic diagram depicting a device for measuring the zeta potential of a filtration medium.

Figure 6 is a graph of filtration medium zeta potential (mV) versus electrolyte solution pH, wherein curve "a" is for a new porous membrane, curve "b" is for a porous membrane that has been partially clogged in connection with filtering beer, and curve "c" is for a porous membrane that has been nearly fully clogged in connection with filtering beer.

Figure 7 is a schematic diagram depicting an apparatus for filtering beer using a bypass system and the measuring device of Fig. 5.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a method for producing beer, preferably cold-filtered beer. The method comprises filtering beer through a porous membrane, i.e., a membrane filter, until such time that the porous membrane is in need of cleaning, contacting the porous membrane with an enzyme to clean the porous membrane, and then reusing the porous membrane to continue filtering beer.

It surprisingly has been discovered that porous membranes can be cleaned better and more gently with a cellulase and/or with an amylase than with proteases, xylanases, and/or glucanases. Cleaning in accordance with the present invention results in a considerable increase in the service life of porous membranes used in the filtering of beer and therefore greatly improves the commercial benefit attendant the use of porous membranes in the production of beer.

The enzyme is selected from the group consisting of cellulases, amylases, and combinations thereof. As indicated above, proteases, xylanases, and/or glucanases need not be used, and, preferably, are not used, with the cellulase and/or amylase to clean the porous membrane. The cellulase desirably has a crystalline:soluble cellulose activity ratio (described more fully below) of at least about 0.1, more desirably at least about 0.3, preferably at least about 0.4, more preferably at least about 0.5, and most preferably at least about 1, particularly at least about 1.2. Suitable cellulases include cellulases derived from *Aspergillus*, particularly *Aspergillus niger*. Preferred cellulases include cellulases derived from *Trichoderma*, preferably *Trichoderma reesei* and *Trichoderma longibrachiatum*, and

Thermomonospora, preferably from *Thermomonospora fusca*. Other sources of cellulases are recited in U.S. Patent 4,912,056. Suitable amylases include α-amylase, β-amylase, and combinations thereof. More preferably, no enzymes other than cellulases and amylases are utilized in the present inventive method, i.e., the porous membrane is not contacted with an enzyme other than a cellulase or an amylase. Most preferably, the enzyme utilized in the present inventive method is a cellulase, and optimally no enzyme other than a cellulase is utilized, i.e., the porous membrane is contacted with a cellulase and is not contacted with any other enzyme.

The porous membrane can be any membrane suitable for the filtration of beer. In the context of the present invention, the porous membrane typically will be a microporous membrane, i.e., a porous membrane with a pore rating of about 0.02-1 μm. The porous membrane preferably will have a pore rating of about 0.1-1 μm, most preferably about 0.45 μm. Such a porous membrane can be used to remove bacteria and other undesirable germs from the beer, preferably obviating the need to pasteurize the beer. The porous membrane also can be used to remove yeast and other undesirable substances from the beer. Suitable porous membranes include those prepared from inorganic materials such as ceramics and metals, as well as, preferably, organic polymers such as polyamides, polyethersulfones, polyolefins, polyvinylidenefluoride, and the like. The porous membrane preferably is a polyamide porous membrane, especially a nylon-6,6 porous membrane.

A preferred embodiment of the method according to the present invention is characterized in that the porous membrane is additionally brought into contact with an aqueous base, with the porous membrane being advantageously brought into contact with the aqueous base at a first stage and with the enzyme at a second stage. The use of an aqueous solution of NaOH and/or KOH as the

aqueous base has proven expedient. It is preferable for the base to be present in a concentration of 0.1 to 1 N, more preferably 0.25 to 1 N, and most preferably 0.5 to 1 N. The treatment with the aqueous base is best carried out at a temperature of between 40 and 90 °C.

Further advantageous embodiments of the method according to the present invention are characterized in that the treatment with the cellulase is carried out at a temperature of between 40 and 50 °C and a pH of between 4.5 and 5.5, the treatment with the α-amylase is carried out at a temperature of between 60 and 75 °C and a pH of between 4.6 and 5.8, and the treatment with the β-amylase is carried out at a temperature of between 40 and 60 °C and a pH of between 4.6 and 5.8.

It is expedient for the cleaning to be carried out until a point in time at which there is no more change in the streaming potential or the zeta potential of the porous membrane. It has been discovered that the streaming potential occurring at the porous membrane during operation or the zeta potential calculated from it (see below) is a good indication of the extent to which the substances clogging the porous membrane have been removed.

The present invention also aims at increasing the porous membrane's service life by ensuring that it is cleaned at a desirable time. Thus, the present invention provides for the production of beer comprising filtering beer through a porous membrane, which will clog progressively as filtration proceeds. Filtration is halted at a given point when the porous membrane is only partially clogged, i.e., has not yet reached the condition of being totally clogged. The degree of clogging can be determined by any suitable means, desirably by monitoring the pressure drop across the porous membrane such as is generally described in U.S. Patent 5,449,465. Alternatively, the present invention provides for an identification of the time for cleaning

by determination of the streaming potential through the filter and/or zeta potential of the filter.

This aspect of the present invention is founded on the recognition that streaming potential -- or zeta potential extrapolated from the former's recorded data -- will change in a pH range (within which beer brewing or filtering occurs) according to the degree of clogging and thus represents a reliable, and almost quantitative, indicator of the state of clogging. Determination of the streaming potential and/or zeta potential of the porous membrane can hence give an accurate picture of a particular state of clogging.

Porous membranes are known to act in a two-fold way. First, a porous membrane acts as a sieve, when particles larger then the filter's pores are mechanically filtered out of the medium. Secondarily, a porous membrane also is known to act by electrostatic attraction. Particles of a diameter much smaller than the pore size of the membrane are deposited thereon when the zeta potential of the filter medium and that of the particles are of opposite polarity (see, e.g., Informational Brochure SD 872h G of Pall Filtrationstechnik GmbH, Germany).

Yet, not known prior to the present invention is the fact that zeta potential can be used to determine a porous membrane's degree of clogging.

A porous membrane's zeta potential will be affected by its chemical properties. The expert will have no difficulty -- being cognizant of the present invention -- to select only filters whose zeta potential will change at a great enough rate relative to the degree of clogging. With the filter on-line, and by way of continuous monitoring through data acquisition, the filtration process can be halted at an appropriate time, e.g., once clogging sets in.

The cleaning of a filter not yet fully clogged is much easier, while assuring longer service life, than the cleaning of a totally clogged filter. Thus, a preferred

method of the present invention has filtration halted at a point when the filter's zeta potential has decreased to a maximum of 20% of the value it exhibited in its unused state, or when clogging does not exceed 80%.

Another refinement of the process will use a porous membrane of polyamide, with filtration halted when the zeta potential exceeds -5 mV as measured at a pH of 4.2.

The beer preferably will undergo pre-filtration before filtration proper, i.e., filtration through the porous membrane. Diatomateous (or infusorial) earth, also known as diatomite, is almost exclusively used for pre-filtration. A combination of diatomateous earth and deep-bed filtration also is feasible.

The present invention can be used in any suitable beer production system. Preferably, the present invention is used in connection with the cluster filter system as described in U.S. Patents 5,417,101 and 5,594,161.

The present invention also relates to a filtration unit for filtering beer, with a feeder line for the filtration-bound beer, a porous membrane, and a run-off line for the filtered beer. It is characterized by a module in the form of a meter cell, functioning as bypass, and featuring a porous membrane and means, e.g., electrodes, for monitoring the streaming potential and/or zeta potential of the meter cell's membrane filter through which beer flows.

The present invention also deals with a filtration unit for filtering beer, with the unit featuring a feeder line for filtration-bound beer, a porous membrane, and a run-off line for filtered beer. In divergence from the foregoing paragraph, the filtration unit is characterized by means, e.g., electrodes, being attached to the porous membrane for monitoring or reading the streaming potential and/or zeta potential as the beer flows through the porous membrane. In this variation, the zeta potential is not measured via the meter cell assigned as

bypass to the membrane filter, but rather on the membrane filter itself.

Any suitable bypass configuration can be utilized in connection with the embodiments of the present invention. Preferably, the present invention incorporates the apparatus and method described in U.S. Patent 5,449,465.

The discovery that the filter's zeta potential correlates to the general state of clogging can be implemented in beer filtration as follows:

1. Through constant observation of changes taking place in the streaming potential and/or zeta potential of the porous membrane during the filtration process, the membrane's degree of clogging can be pinpointed in order to prevent an unexpected or random occurrence, while timely measures for an exchange of filters can be taken.

2. Filtration can be halted before the porous membrane becomes totally clogged. This promotes easier cleaning of the filter. It has been shown that the clogging substances in a totally clogged filter can only be removed with the greatest of difficulty by conventional methods of cleansing, or cannot be removed from the filter at all, resulting in abbreviated service life.

Once filtration is halted prior to total clogging, the process of cleaning is much easier and more thorough, with the filter retaining an extended life. In the instance of a polyamide porous membrane, it has been discovered that the successful removal of all clogging substances from the porous membrane can be accomplished when filtration is halted at a point where the zeta potential has not lost more than about 80% of its original value, i.e., is not clogged in excess of 80%.

3. The cleaning method's success can be tested by determining the cleaned membrane's zeta potential. The act of cleaning will return the zeta potential to approximately its original value.

By this procedure, the cleaning process can be evaluated and/or optimized for it's efficiency:

4. The aging of a porous membrane for reasons of repeated use can be tracked, providing a handy estimate as to its remaining service life expectancy.

5. By measuring zeta potential, filter material and shunting materials (e.g., diatomite, bentonite, perlite, polyvinyl pyrrolidone) can be tested for suitability in beer filtration by assessing the interaction between clogging substances of liquid systems and filter material and/or shunting means for filters.

6. The service life of a porous membrane can be estimated by way of measuring zeta potential, whereunder a specific membrane load (hl/m^2) is recorded up to the point when clogging sets in.

The artisan is aware that most suitable for the process are porous membranes with a zeta potential exhibiting pronounced change in relation to the degree of clogging. Verification of these parameters is easy enough by employing the aforementioned simple test method.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

This example illustrates the effectiveness of the present inventive method to produce beer. In particular, this example demonstrates that cellulases and amylases can be used to satisfactorily clean a porous membrane clogged in the course of beer filtration such that the porous membrane can be reused in continued beer filtration.

A porous membrane made of nylon-6,6 (NB type, commercially available from Pall Filtrationstechnik GmbH, Germany) was used as a filter. Such a filter is frequently used in the state of the art for the cold-filtration of beer.

The so-called membrane filter test according to Esser (Monatszeitschrift für Brauerei (Monthly Magazine for Breweries), 25th year, No. 6, pages 145-151, 1972) was used to determine the filtering performance of the filter. This test is reliable for checking measures for improving filterability.

To determine the filtering efficiency of a new, i.e., unused, porous membrane, a pressure filtration apparatus (SM 16526 type, 200 ml capacity; commercially available from Sartorius GmbH, Goettingen, Germany) was used for a polyamide nylon-6,6 porous membrane having a 47 mm diameter and a 0.2 µm pore size.

Beer cooled down to 0 °C was forced through the porous membrane under isobaric conditions (1 bar), and the amount of filtrate was weighed every 10 seconds. The test was stopped after 200 g of filtrate were obtained. The result is shown as a graph in the diagram of Figure 1. Figure 1 shows that, under the conditions indicated above, the 200 g of filtrate were obtained with the unused filter after approximately 210 seconds.

Under identical conditions, the filtering performance of a clogged, i.e., used, porous membrane was tested. The result is given in Figure 2 which shows that even in 720 seconds only approximately 60 g of filtrate were obtained.

The clogged porous membrane was cleaned in accordance with a prior art method, wherein the membrane was first cleaned enzymatically and then chemically, as described below.

For enzymatic cleaning, the clogged membrane was treated for 1 hour with a 1% aqueous solution of a mixture of β-glucanases and xylanases (P3-Ultrasil 65; commercially available from Henkel) with a pH of 5 (adjusted with a 0.05% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel)) at a temperature of 50 °C. This treatment was subsequently carried out one more time.

The membrane was then treated for 3 hours with a 0.5% aqueous solution of a mixture of surfactants, glucanases, and proteases (P3-Ultrasil 62; commercially available from Henkel) with a pH of 9-9.5 (set with a 0.15% aqueous solution of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; commercially available from Henkel)) at a temperature of 50 °C and subsequently rinsed with warm water (50 °C).

For chemical cleaning, the membrane thereafter was treated for 30 minutes with a 1% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel) at 60 °C, and then rinsed with fresh water. The membrane was subsequently treated for 30 minutes with an aqueous solution containing 1% of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; commercially available from Henkel) and 1% of a mixture of surfactants and an oxygen donor (P3-Ultrasil 05; commercially available from Henkel) at a temperature of 60 °C and then rinsed with fresh water. The membrane was then treated once more for 30 minutes with a 0.5% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel) and subsequently rinsed with fresh water until the rinse water reached the electrical conductivity of fresh water.

The filtering performance of this cleaned porous membrane was then tested again under the conditions indicated above. The result is shown in Figure 3. Figure 3 shows that the filtering performance has improved somewhat as the 200 g of filtrate were obtained after approximately 600 seconds.

A similarly clogged membrane whose filtering efficiency is shown in Figure 2 was cleaned in accordance with the method according to the present invention. The membrane was treated for 30 minutes with an aqueous solution of C_1- and C_x-cellulases, the solution having a pH value of 4.7, at a temperature of 45 °C. The membrane was then treated with the same solution, but at a pH value of 5.0 and a

temperature of 50 °C, and, finally, at a pH value of 4.7 and a temperature of 60 °C for 60 minutes.

The membrane was subsequently rinsed with warm water at 50 °C. The filtering performance of the membrane cleaned in accordance with the present invention was tested in accordance with the above procedure. The result is shown in Figure 4.

Figure 4 shows that 200 g of filtrate were obtained after approximately 220 seconds. This represents a significant improvement over the prior art (Figure 3). The method according to the present invention, therefore, allows considerably better cleaning of a used membrane filter than is possible with prior art cleaning methods.

Equally good results were obtained when, in accordance with the present invention, an amylase was used instead of a cellulase. The service life of a porous membrane thus can be increased with the cleaning method according to the present invention.

EXAMPLE 2

This example illustrates the use of the streaming or zeta potential of a porous membrane to assist in the cleaning of the porous membrane. In particular, the streaming or zeta potential is demonstrated to be useful in determining the extent of membrane cleaning as well as when a membrane is most satisfactorily cleaned.

The zeta potential of membrane filters was determined with the electrokinetic measuring system EKA of Anton Paar GmbH, Austria. This measurement is based on the streaming potential method. An electrolyte flows through the filters, and the potential (streaming potential) which is produced by shearing-off of counterions is detected with electrodes, and the zeta potential is calculated from this measured quantity (see below).

Figure 5 shows diagrammatically the measuring cell with which the streaming potential or the zeta potential

was determined. Reference numeral 1 designates the measuring cell in which the porous membrane 2 is clamped without warping in filter holders 3 and 4 made of polytetrafluoroethylene. The filter holders 3 and 4 are the end pieces of two pistons 5 and 6, respectively, which are mounted for displacement in the cylindrical part 7 of the measuring cell 1.

The end pieces 3 and 4 of the pistons 5 and 6, respectively, have fine bores 10 and 11 for the fluid which is to be filtered and press the perforated electrodes 8 and 9 against the porous membrane 2. The electrodes 8 and 9 are connected to the two electric terminals 12 and 13 extending inside the pistons 5 and 6 so the streaming potential built up as fluid flows through the membrane 2 can be measured. Silver electrodes or silver chloride electrodes which exhibit a low polarization during passage of current are preferred for the electrodes. The pistons 6 and 7 are mounted in the seals 14 and 15, respectively, such that, on the one hand, they are displaceable, and, on the other hand, they do not allow any fluid to leak from the measuring cell.

The fluid to be filtered flows through the supply line 16 into the cylindrical part 7 of the measuring cell 1, through the fine bores 10 of the piston 6, through the electrode 8, with an electric potential being built up, and through the porous membrane 2. The filtered fluid flows through the electrode 9, with a potential likewise being built up, passes through the fine bores 11 of the piston and leaves the measuring cell through the discharge line 17.

To determine the zeta potential from the measured streaming potential, measurement (not illustrated) of the differential pressure in the measuring cell between supply line 16 and discharge line 17, the conductivity and also the pH value is necessary. The zeta potential is calculated from these measured quantities as follows:

$$\text{zeta potential} = \frac{U}{\Delta p} \cdot \frac{LF.\eta}{\varepsilon.\varepsilon_0}$$

where U is the streaming potential, Δp the pressure difference, LF the conductivity, η the viscosity, and $\varepsilon\varepsilon_0$ the dielectric constant.

The change in the zeta potential of the membrane filter as clogging progresses is shown in Figure 6. This figure is a diagram in which the zeta potential in millivolts is plotted as ordinate, and the pH value at which the zeta potential was determined as abscissa. The pH value of the electrolyte solution (0.001 N aqueous KCl solution) was set with 0.1 N HCl or with 0.1 N NaOH. The specified pressure difference was 350 mbar.

The diagram was obtained by first determining with the measuring cell described above the zeta potentials of a new, i.e., unused porous membrane made of polyamide (NB type, commercially available from Pall Filtrationstechnik GmbH, 6072 Dreieich 1, Germany) at various pH values.

The results relating to the unused porous membrane are plotted as curve "a". It is evident that the unused porous membrane has a zeta potential of approximately -18 mV with an alkaline pH, and that the zeta potential increases with decreasing pH and finally reaches zero value at a pH of approximately 3.

Curve "b" shows the dependence of the zeta potential on the pH value of the porous membrane under identical measuring conditions, as stated above, but after use thereof for filtering beer and, therefore, with partial clogging. As is apparent, the zeta potential is raised somewhat by the partial clogging and only reaches a value of approximately -15 mV at pH values of approximately 7.

Curve "c" was plotted for the same porous membrane in the nearly fully clogged state. It is evident that the zeta potential now changes only slightly with the pH value, and even in the alkaline range does not fall below approximately -2 mV.

To test the cleaning according to the present invention, the zeta potential of the membrane to be cleaned is determined, and the cleaning was successful if the zeta potential of the cleaned membrane shifted as far as possible in the direction of the zeta potential of the unused membrane.

It will be clear to one skilled in the art that porous membranes whose zeta potential changes to a sufficiently great extent as a function of the degree of clogging are particularly well-suited for use in the method according to the present invention. This characteristic can be easily determined by one skilled in the art by simple testing.

A porous membrane of polyamide is especially suitable in the context of the process since the zeta potential at the pH of the filtration-bound beer (ca. pH = 4.2) will undergo severe change with progressive clogging. As can be learned from Figure 6, the membrane at this particular pH value at the beginning of filtration shows a zeta potential of approximately -8 mV. The totally clogged membrane has a zeta potential of approximately -2 mV.

Figure 7 shows a variation of the discussed filtration unit featuring a filtration chamber 18, with a meter cell 22 assigned to it as a bypass, as depicted in Figure 5. The filtration chamber 18 holds filter candles 19.

The filtration-bound beer is fed via line 20 into the filtration chamber 18, flowing through the filter candles (membrane filter) 19, and exits the filter chamber 18 through run-off line 21 in the form of filtered beer.

The meter cell is shown in Figure 7 without detail. The actual flow through the meter cell 22 must be controlled to the extent that an amount of beer is filtered per cm^2 of the porous membrane's surface which is

equal to the amount of porous membrane surface per cm^2 in the filtration chamber 18.

The severe change in filter test membrane 2 (Figure 5) zeta potential inside meter cell 1 during filtration allows an assessment of the state of the filter candles 19 in filtration chamber 18.

EXAMPLE 3

This example illustrates the effectiveness of cellulase derived from *Aspergillus niger* in enzymatically degrading soluble and crystalline cellulose substrates.

Cellulase derived from *Aspergillus niger* was obtained from Fluka (item numbers 22178). The enzyme was evaluated with respect to two different celluloses: soluble carboxymethylcellulose (CMC, available from Aldrich as item number 41927-3) and crystalline cellulose (Avicel, available from FMC as item number PH-105).

The test methodology involved the preparation of an incubation solution of (i) 18 ml CMC (1%) or Avicel (1%), (ii) 5 ml sodium acetate buffer (50 mM, pH 4.8), and (iii) 5 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 4.8) at 30 °C. A test solution then was prepared by mixing 1.4 ml of the incubation solution with 0.1 ml glucose solution (0.15%) and 1.5 ml 3,5-dinitrosalicylic acid (DNS) reagent (available from Sigma as item number D-0550). The test solution was boiled for 15 minutes. The total µmol glucose equivalents/mg enzyme as a function of time (min) was determined spectroscopically (575 nm), using two parallel samples, in accordance with the procedure described in Miller, *Anal. Chem.*, *31*, 426-28 (1959), using a straight line calibration with a glucose standard. Protein amounts were determined in accordance with the procedure described in Bradford, *Anal. Biochem.*, *72*, 248-64 (1976), using a bovine serum albumin (BSA) standard.

The enzymatic degradation of cellulose results in the production of glucose, and, therefore, the measurement of

μmol glucose equivalents/mg enzyme is a measure of the activity of the enzyme with respect to a particular type of cellulose, e.g., soluble (CMC) or crystalline (Avicel) cellulose.

The results of this evaluation with respect to the cellulase derived from *Aspergillus niger* are set forth in Table 1. The test solution with the soluble (CMC) cellulose substrate contained 0.8 mg enzyme/28 ml incubation solution (ca. 17.6 μg protein). The test solution with the crystalline (Avicel) cellulose substrate contained 0.35 mg enzyme/28 ml incubation solution (ca. 7.7 μg protein).

Table 1: Cellulase derived from *Aspergillus niger*

Time (min)	Glucose Equivalents (μmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
10	27.0	0	0
15	28.5	1.7	0.06
30	30.5	--	--
45	34.0	1.7	0.05
60	34.8	4.0	0.11
75	37.5	3.5	0.09
90	37.8	3.7	0.10
105	38.3	--	--
120	39.5	10.3	0.26

Those enzymes that have a relatively greater activity toward crystalline cellulose substrates as compared to soluble cellulose substrates have been found to be particularly effective in cleaning porous membranes used in beer filtration. The ratio of the glucose equivalents produced with respect to the crystalline cellulose substrate and the glucose equivalents produced with

respect to the soluble cellulose substrate thus is an indicator of the effectiveness of the enzyme in the context of the present invention and is described as the crystalline:soluble cellulose activity ratio. Desirably, the crystalline:soluble cellulose activity ratio has the previously described values at a range of times in the test protocol described in this example, e.g., at 30 minutes, 60 minutes, and/or 90 minutes, especially at 60 minutes.

As is apparent from the data set forth in Table 1, the cellulase from *Aspergillus niger* has a crystalline:soluble cellulose activity ratio at 60 minutes of 0.11, indicating that it is a moderately effective enzyme for purposes of cleaning porous membranes used in connection with the filtration of beer.

EXAMPLE 4

This example illustrates the effectiveness of cellulase derived from *Trichoderma reesei* in enzymatically degrading soluble and crystalline cellulose substrates.

Cellulase derived from *Trichoderma reesei* was obtained from Fluka (item numbers 22173). The enzyme was evaluated in the same manner as recited in Example 3.

The results of this evaluation with respect to the cellulase derived from *Trichoderma reesei* are set forth in Table 2. The test solution with the soluble (CMC) cellulose substrate contained 0.37 mg enzyme/28 ml incubation solution (ca. 128 µg protein). The test solution with the crystalline (Avicel) cellulose substrate contained 0.08 mg enzyme/28 ml incubation solution (ca. 25.6 µg protein).

Table 2: Cellulase derived from *Trichoderma reesei*

Time (min)	Glucose Equivalents (μmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	62.6	0	--
10	84.7	21.5	0.25
15	96.3	30.0	0.31
30	99.5	40.0	0.40
45	152.0	57.5	0.38
60	139.0	75.0	0.54
75	178.4	85.0	0.48
90	184.2	95.0	0.52
105	172.6	100.0	0.58
120	193.7	115.0	0.59

As is apparent from the data set forth in Table 2, the cellulase from *Trichoderma reesei* has a crystalline:soluble cellulose activity ratio at 60 minutes of 0.54, indicating that it is a superior enzyme for purposes of cleaning porous membranes used in connection with the filtration of beer.

EXAMPLE 5

This example illustrates the effectiveness of β-cellulase derived from *Bacillus subtilis* in enzymatically degrading soluble and crystalline cellulose substrates.

β-cellulase derived from *Bacillus subtilis* was obtained from Fluka (item numbers 49106). The enzyme was evaluated in the same manner as recited in Example 3.

The results of this evaluation with respect to the β-cellulase derived from *Bacillus subtilis* are set forth in Table 3. The test solution with the soluble (CMC) cellulose substrate contained 14.4 mg enzyme/28 ml incubation solution (ca. 8.3 μg protein). The test

solution with the crystalline (Avicel) cellulose substrate contained 15.6 mg enzyme/28 ml incubation solution (ca. 8.8 µg protein).

Table 3: β-Cellulase derived from *Bacillus subtilis*

Time (min)	Glucose Equivalents (µmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	1.1	0.1	0.09
10	1.0	0.1	0.10
15	0.9	0.1	0.11
30	0.9	0.1	0.11
45	1.0	0.1	0.10
60	1.0	0.1	0.10
75	1.0	0.2	0.20
90	1.1	0.2	0.18
105	1.1	0.1	0.09
120	1.3	0.1	0.08

As is apparent from the data set forth in Table 3, the β-cellulase from *Bacillus subtilis* has a crystalline:soluble cellulose activity ratio at 60 minutes of 0.10, indicating that it is a moderately effective enzyme for purposes of cleaning porous membranes used in connection with the filtration of beer.

EXAMPLE 6

This example illustrates the effectiveness of exocellulase derived from *Thermomonospora fusca* in enzymatically degrading soluble and crystalline cellulose substrates.

Exocellulase E3 derived from *Thermomonospora fusca* was obtained from Cornell University. The enzyme was evaluated in the same manner as recited in Example 3

except that the incubation solution comprised (i) 18 ml CMC (1%) or Avicel (1%), (ii) 9 ml sodium acetate buffer (50 mM, pH 5.6), and (iii) 1 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 5.6), shaken at 50 °C (ca. 960 μm protein). The test solution was evaluated using a color test rather than the DNS test recited in Example 3.

The results of this evaluation with respect to the exocellulase derived from *Thermomonospora fusca* are set forth in Table 4.

Table 4: Exocellulase derived from *Thermomonospora fusca*

Time (min)	Glucose Equivalents (μmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	0.1	0	--
10	0.1	0.3	3.00
15	0.2	0.3	1.50
30	0.2	0.3	1.50
45	0.3	0.4	1.33
60	0.3	0.4	1.33
75	0.3	0.5	1.67
90	0.3	0.3	1.00

As is apparent from the data set forth in Table 3, the β-cellulase from *Bacillus subtilis* has a crystalline:soluble cellulose activity ratio at 60 minutes of 1.33, indicating that it is a superior enzyme for purposes of cleaning porous membranes used in connection with the filtration of beer.

EXAMPLE 7

This example illustrates the effectiveness of α-amylase derived from *Bacillus subtilis* in enzymatically degrading soluble and crystalline cellulose substrates.

α-amylase derived from *Bacillus subtilis* was obtained from Fluka (item numbers 10069). The enzyme was evaluated in the same manner as recited in Example 3 except that the incubation solution comprised (i) 18 ml CMC (1%) or Avicel (1%), (ii) 5 ml sodium acetate buffer (50 mM, pH 6.9), and (iii) 5 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 6.9), shaken at 30 °C (ca. 8.5 μm protein). The test solution was evaluated using a color test rather than the DNS test recited in Example 3.

The results of this evaluation with respect to the α-amylase derived from *Bacillus subtilis* are set forth in Table 5.

Table 5: α-Amylase derived from *Bacillus subtilis*

Time (min)	Glucose Equivalents (μmol/mg enzyme)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--
5	0.1	0	0
10	0.1	0	0
15	0.1	0	0
30	0.1	0	0
45	0.1	0	0
60	0.1	0	0
75	0.1	0	0
90	--	0	--

As is apparent from the data set forth in Table 5, the α-amylase from *Bacillus subtilis* has a crystalline:soluble cellulose activity ratio at 60 minutes of about 0 (<0.1 μmol detection limit), indicating that it

is not as effective an enzyme for purposes of cleaning porous membranes used in connection with the filtration of beer as the previously described cellulases.

EXAMPLE 8

This example illustrates the effectiveness of various cellulases in enzymatically degrading soluble and crystalline cellulose substrates.

Cellulase preparations were obtained from the Erbsloh Company: (a) C_x-cellulase (powder, item number VP 0945/2), (b) C_1-cellulase from *Trichoderma reesei* (powder, item number VP 0965/2), (c) C_1-cellulase (liquid, item number Cleanzym SB1), (d) C_1-cellulase (liquid, item number VP 0976/4), (e) cellulase (liquid, item number VP 0971/1), and (f) cellulase (liquid, item number VP 0971/4). The enzymes were evaluated in a manner similar to that recited in Example 3 except that the incubation solutions comprised (i) 23 ml CMC (1%) or Avicel (1%) in a sodium acetate buffer (50 mM, pH 4.8), and (ii) 5 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 4.8). 0.5% stock solutions were prepared from the powdered enzyme preparations (5 mg/ml) and liquid enzyme preparations (5 µl/ml). The solutions were shaken at 30 °C. The test solution was evaluated after making a 1:5 dilution using a color test rather than the DNS test recited in Example 3.

The results of this evaluation with respect to the various cellulases are set forth in Table 6. The glucose equivalents data is in terms of average µmol glucose equivalents/min (for the total time interval) and are not normalized per mg enzyme (as was the situation with the data recited in Tables 1-5). The crystalline:soluble cellulose activity ratio, of course, is not altered by the units for the glucose equivalents inasmuch as the units divide out in calculating the ratio (i.e., the ratio is unit-less).

Table 6: Cellulase Preparations

Time (min)	Glucose Equivalents (μmol/min)								
	Preparation (a)			Preparation (b)			Preparation (c)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--	0	0	--	0	0	--
5	0.12	0	0	0.14	0.11	0.79	0.09	0.05	0.56
10	0.06	0.01	0.17	0.11	0.08	0.73	0.09	0.06	0.67
15	0.06	0.01	0.17	0.08	0.09	1.13	0.08	0.04	0.50
30	0.02	0.02	1.00	0.07	0.05	0.71	0.07	0.03	0.43
45	0.04	0.02	0.50	0.06	0.04	0.67	0.03	0.02	0.67
60	0.03	0.02	0.67	0.04	0.03	0.75	0.05	0.02	0.40
75	0.03	0.02	0.67	0.04	0.03	0.75	0.04		--
90	0.02	0.01	0.50	0.03	0.03	1.00	0.03	0.2	0.67

Time (min)	Glucose Equivalents (μmol/min)								
	Preparation (d)			Preparation (e)			Preparation (f)		
	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio	Soluble Cellulose Substrate	Crystalline Cellulose Substrate	Crystalline: Soluble Cellulose Activity Ratio
0	0	0	--	0	0	--	0	0	--
5	0.01	0.23	23.0	0.15	0.13	0.87	0.07	0.04	0.57
10	0.19	0.14	0.74	0.10	0.09	0.90	0.06	0.03	0.50
15	0.15	0.12	0.80	0.06	0.05	0.83	0.04	0.02	0.50
30	0.10	0.09	0.90	0.05	0.03	0.60	0.03	0.01	0.33
45	0.07	0.07	1.00	0.04	0.03	0.75	0.02	0.01	0.50
60	0.06	0.06	1.00	0.03	0.02	0.67	0.02	0.01	0.50
75	0.05	0.06	1.20	0.03	0.02	0.67	0.02	0.01	0.50
90	0.04	0.06	1.50	0.03	0.02	0.67	0.01	0.01	1.00

As is apparent from the data set forth in Table 6, the various cellulases have crystalline:soluble cellulose activity ratios at 60 minutes ranging from 0.4-1.0, indicating that they are superior enzymes for the purpose of cleaning porous membranes used in connection with the filtration of beer.

EXAMPLE 9

This example further illustrates the effectiveness of the present inventive method to produce beer. In particular, this example demonstrates that cellulases alone (i.e., without the use of other enzymes) are superior in the cleaning of porous membranes clogged in the course of beer filtration for the purpose of returning the porous membrane to use in continued beer filtration.

Beer of different characteristics was filtered through nylon-6,6 porous membranes (ca. 300 m^2) with a pore rating of 0.45 μm in a cluster filter arrangement (PALL-CFS, available from Pall Filtrationstechnik GmbH, Germany). At certain beer filtration intervals, the porous membranes were subjected to a cleaning process in accordance with the present invention.

The cleaning process involved circulation of a 0.5% NaOH solution for 15 minutes, followed by a 60 minute soak. The porous membranes then were backflushed with water. An internal loop was established through the cluster filter arrangement with water at 38 °C. Lactic acid was added to the water to adjust the pH to 4.2 ± 0.3, and then 6 l of an enzyme preparation containing a cellulase derived from *Trichoderma longibrachiatum* obtained from the Erbsloh Company (item number VP 0945/1) was added to the water via a dosing pump. The enzyme preparation in the water (at a concentration of about 20-40 g enzyme/100 kg filter housing fluid volume) was circulated for about 15 minutes, followed by a 30 minute

soak, another 15 minute circulation, and finally a 6 hour soak. The porous membranes then were backflushed with water.

The porous membranes were cleaned after about 90,000 hl total beer was filtered through the porous membranes, and then the porous membranes were returned to service, i.e., to continue filtering beer. The porous membranes similarly were cleaned and returned to service after about 100,000 hl, about 140,000 hl, and about 165,000 hl total beer was filtered through the porous membranes. The porous membranes mechanically failed after about 190,000 hl total beer was filtered through the porous membranes.

The foregoing data demonstrates that beer can be satisfactorily produced using the present invention. Specifically, the results of this example demonstrate that a porous membrane can be effectively cleaned and returned to service in accordance with the present invention, thereby prolonging the useful life of the porous medium in a beer production process.

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A method for producing beer comprising filtering beer through a porous membrane until such time that said porous membrane is in need of cleaning, contacting said porous membrane with an enzyme selected from the group consisting of cellulases, amylases, and combinations thereof in the absence of a protease or a glucanase to clean said porous membrane, and then reusing said porous membrane to continue filtering beer.

2. The method of claim 1, wherein said porous membrane is not contacted with an enzyme other than said cellulase or said amylase.

3. The method of claim 1 or 2, wherein said porous membrane is contacted with said cellulase.

4. A method for producing beer comprising filtering beer through a porous membrane until such time that said porous membrane is in need of cleaning, contacting said porous membrane with a cellulase having a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.1 to clean said porous membrane, and then reusing said porous membrane to continue filtering beer.

5. The method of claim 3 or 4, wherein said porous membrane is contacted with said cellulase and is not contacted with any other enzyme.

6. The method of any of claims 1-3 or 5, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.1.

7. The method of claim 6, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.3.

8. The method of claim 7, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.4.

9. The method of claim 8, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.5.

10. The method of claim 9, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 1.

11. The method of claim 10, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 1.2.

12. The method of any of claims 1-11, wherein said cellulase is derived from *Trichoderma*.

13. The method of claim 12, wherein said *Trichoderma* is *Trichoderma reesei* or *Trichoderma longibrachiatum*.

14. The method of any of claims 1-11, wherein said cellulase is derived from *Thermomonospora*.

15. The method of claim 14, wherein said *Thermomonospora* is *Thermomonospora fusca*.

16. The method of any of claims 1-3 and 6-15, wherein said porous membrane is contacted with said amylase.

17. The method of claim 16, wherein said amylase is selected from the group consisting of α-amylase, β-amylase, and the combination thereof.

18. The method of any of claims 1-17, wherein said porous membrane is additionally contacted with an aqueous base prior to being reused.

19. The method of claim 18, wherein said porous membrane is contacted with said aqueous base prior to being contacted with said enzyme.

20. The method of claim 18 or 19, wherein said aqueous base is an aqueous solution of NaOH and/or KOH.

21. The method of any of claims 18-20, wherein said base is present in a concentration of 0.1-1 N in said aqueous base.

22. The method of any of claims 18-21, wherein said porous membrane is contacted with said aqueous base at a temperature of 40-90 °C.

23. The method of any of claims 1-22, wherein said porous membrane is contacted with said cellulase at a temperature of 40-50 °C and a pH of 4.5-5.5.

24. The method of any of claims 1-3 and 6-23, wherein said porous membrane is contacted with α-amylase at a temperature of 60-75 °C and a pH of 4.6-5.8.

25. The method of any of claims 1-3 and 6-23, wherein said porous membrane is contacted with β-amylase at a temperature of 40-60 °C and a pH of 4.6-5.8.

26. The method of any of claims 1-25, wherein said porous membrane is cleaned until the zeta potential of said porous membrane ceases to change.

27. The method of any of claims 1-26, wherein said time that said porous membrane is in need of cleaning is determined by the pressure drop across said porous membrane.

28. The method of any of claims 1-26, wherein said time that said porous membrane is in need of cleaning is determined by the streaming or zeta potential of said porous membrane.

29. A method for producing beer comprising filtering beer through a porous membrane that progressively clogs during filtration, monitoring the streaming or zeta potential of said porous membrane as a measure of the extent of clogging of said porous membrane, halting filtration of the beer through said porous membrane before said porous membrane becomes fully clogged as determined by the streaming or zeta potential of said porous membrane, cleaning said porous membrane, and then reusing said porous membrane to continue filtering beer.

30. The method of claim 28 or 29, wherein said filtration is halted when the streaming or zeta potential

of said porous membrane is reduced to 20% of its original value for the unused porous membrane.

31. The method of any of claims 1-30, wherein said porous membrane is a polyamide porous membrane.

32. The method of claim 31, wherein said filtration is halted when the zeta potential of said porous membrane exceeds -5 mV as measured at pH 4.2.

33. The method of any of claims 1-32, wherein said filtering beer is cold-filtering beer.

34. A filtration unit for filtering beer comprising a feeder line for the filtration-bound beer, a porous membrane, a run-off line for the filtered beer, and means for monitoring the streaming potential and/or zeta potential of said porous membrane through which beer flows.

35. The filtration unit of claim 34, further comprising a bypass porous membrane through which beer flows, wherein said monitoring means for monitoring the streaming potential and/or zeta potential does so with respect to said bypass porous membrane.

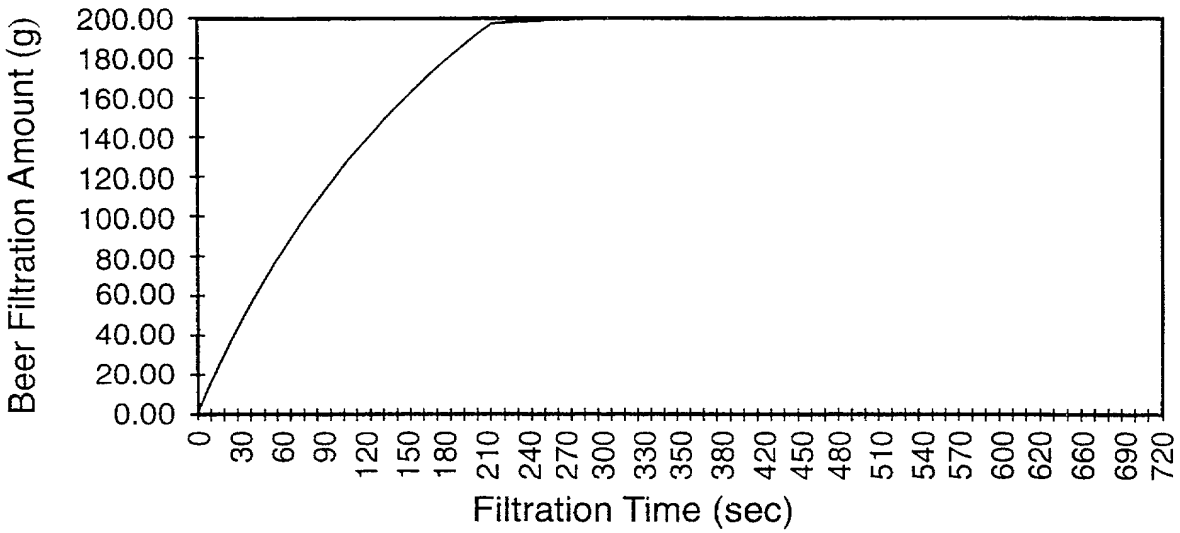

Beer Filtration Amount (g)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
30
60
90
120
150
180
210
240
270
300
330
350
380
420
450
480
510
540
570
600
620
660
690
720
Filtration Time (sec)

FIG. 1

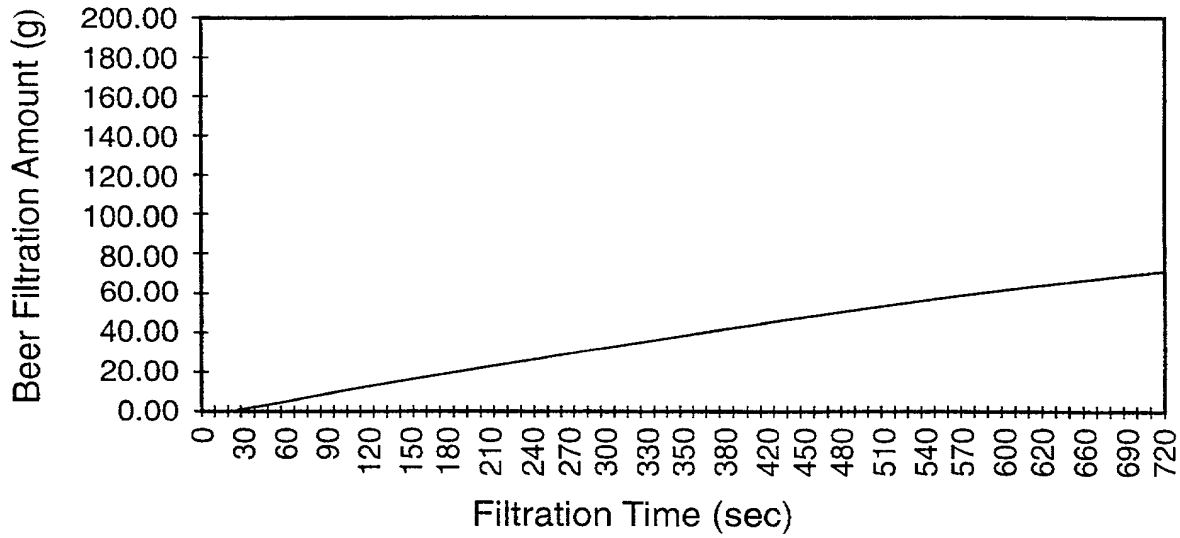

Beer Filtration Amount (g)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
30
60
90
120
150
180
210
240
270
300
330
350
380
420
450
480
510
540
570
600
620
660
690
720
Filtration Time (sec)

FIG. 2

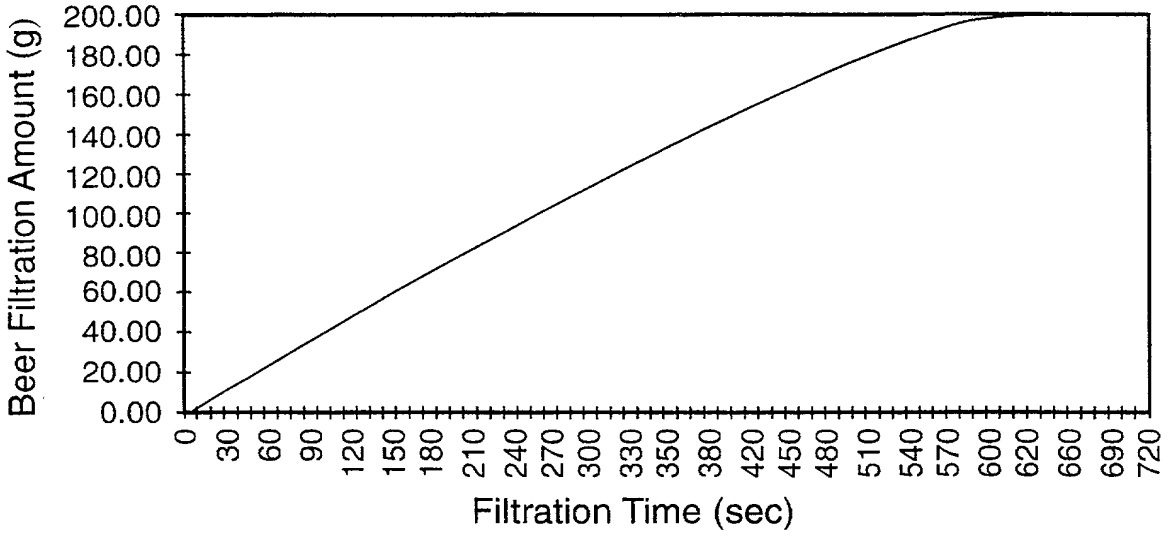

Beer Filtration Amount (g)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
30
60
90
120
150
180
210
240
270
300
330
350
380
420
450
480
510
540
570
600
620
660
690
720
Filtration Time (sec)

FIG. 3

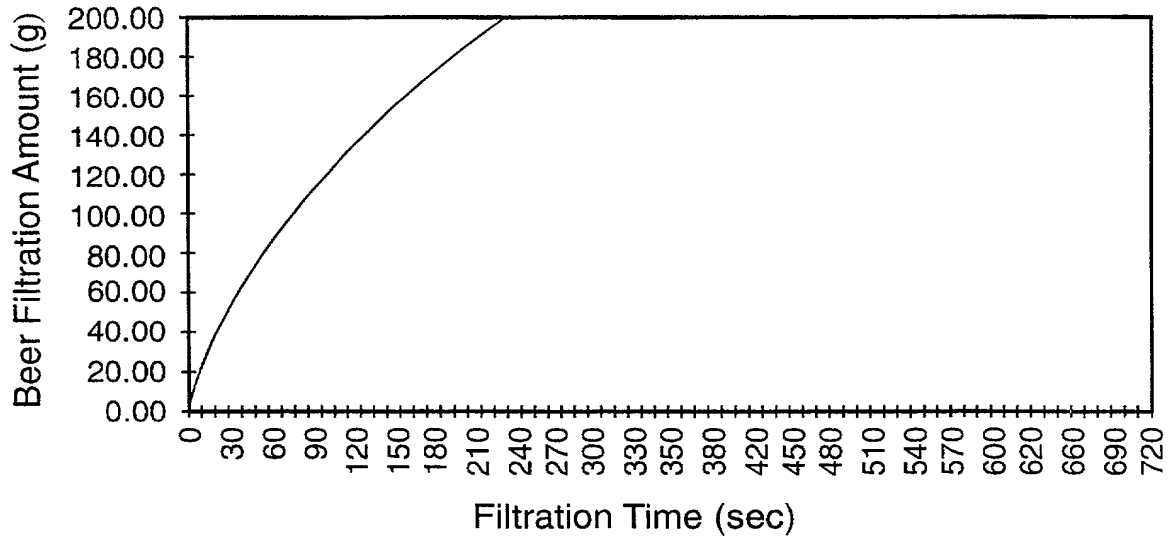

Beer Filtration Amount (g)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
30
60
90
120
150
180
210
240
270
300
330
350
380
420
450
480
510
540
570
600
620
660
690
720
Filtration Time (sec)

FIG. 4

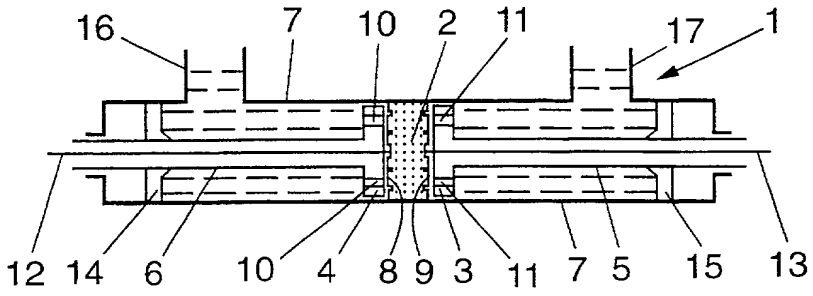

16
7
10
2
11
17
1
12
14
6
10
4
8
9
3
11
7
5
15
13

FIG. 5

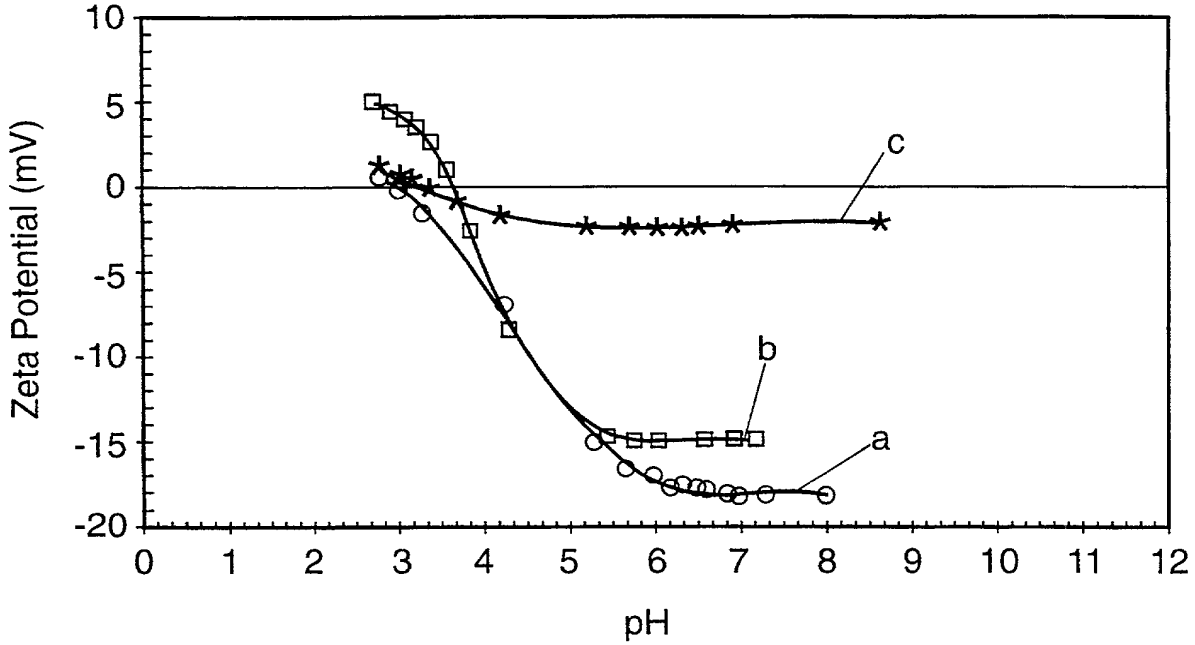

Zeta Potential (mV)
10
5
0
-5
-10
-15
-20
0
1
2
3
4
5
6
7
8
9
10
11
12
pH
a
b
c

FIG. 6

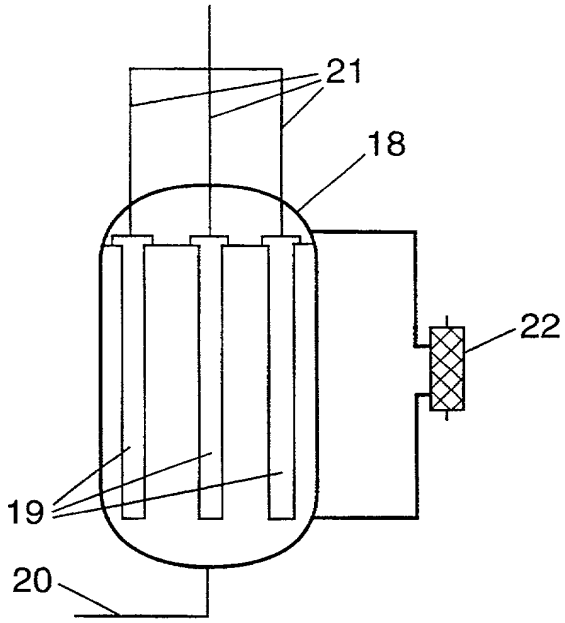


FIG. 7

PATENT
Attorney's Docket No. 202531

COMBINED DECLARATION AND POWER OF ATTORNEY

As below named inventor, I hereby declare that

This declaration is of the following type:

- ☐ original ☐ design ☐ supplemental
- ☒ national stage of PCT
- ☐ divisional ☐ continuation ☐ continuation-in-part

My residence, post office address, and citizenship are as stated below next to my name. I believe I am the original, first, and sole inventor *(if only one name is listed below)* or an original, first, and joint inventor *(if plural names are listed below)* of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR PRODUCING BEER

the specification of which:

- ☐ is attached hereto.
- ☒ was filed on October 8, 1999, as Serial No. 09/402,721 and was amended on _____*(if applicable)*.
- ☐ was filed by Express Mail No. *as Serial No. not known yet*, and was amended on _____*(if applicable)*.
- ☐ was described and claimed in PCT International Application No. _____ filed on_____ and as amended under PCT Article 19 on _____*(if any)*.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

COUNTRY	APPLICATION	DATE OF FILING (day,**month**,year)	PRIORITY CLAIMED UNDER 35 USC 119			
Austria	A596/97	08.04.97	X	YES		NO
Austria	A597/97	08.04.97	X	YES		NO
				YES		NO

I hereby claim the benefit pursuant to Title 35, United States Code, § 119(e) of the following United States provisional application(s):

PRIOR U.S. PROVISIONAL APPLICATIONS CLAIMING THE BENEFIT UNDER 35 USC 119(e)	
APPLICATION NO.	DATE OF FILING

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 USC 120					
U.S. APPLICATIONS			Status *(check one)*		
U.S. APPLICATIONS	U.S. FILING DATE		PATENTED	PENDING	ABANDONED
1. 0 /					
2. 0 /					
3. 0 /					
PCT APPLICATIONS DESIGNATING THE U.S.			Status *(check one)*		
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NOS. ASSIGNED (if any)	PATENTED	PENDING	ABANDONED
4. PCT/US98/06969	07.04.98			X	
5.					
6.					

DETAILS OF FOREIGN APPLICATIONS FROM WHICH PRIORITY CLAIMED UNDER 35 USC 119 FOR ABOVE LISTED U.S./PCT APPLICATIONS				
ABOVE APPLN. NO.	COUNTRY	APPLICATION NO.	DATE OF FILING (day,**month**,yr)	DATE OF ISSUE (day,**month**,yr)
1.				
2.				
3.				
4. PCT/US98/06969	Austria Austria	A596/97 A597/97	08.04.97 08.04.97	
5.				
6.				

As a named inventor, I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Berton Scott Sheppard, Reg. 20922
James B. Muskal, Reg. 22797
Dennis R. Schlemmer, Reg. 24703
Gordon R. Coons, Reg. 20821
John E. Rosenquist, Reg. 26356
John W. Kozak, Reg. 25117
Charles S. Oslakovic, Reg. 27583
Mark E. Phelps, Reg. 28461
H. Michael Hartmann, Reg. 28423
Bruce M. Gagala, Reg. 28844
Charles H. Mottier, Reg. 30874
John Kilyk, Jr., Reg. 30763
Robert F. Green, Reg. 27555
John B. Conklin, Reg. 30369
James D. Zalewa, Reg. 27848
John M. Belz, Reg. 30359
Brett A. Hesterberg, Reg. 31837
Jeffrey A. Wyand, Reg. 29458
Paul J. Korniczky, Reg. 32849
Pamela J. Ruschau, Reg. 34242
Steven P. Petersen, 32927
John M. Augustyn, Reg. 33589
Christopher T. Griffith, Reg. 33392
Wesley O. Mueller, Reg. 33976
Jeremy M. Jay, Reg. 33587
Jeffrey B. Burgan, Reg. 35463
Eley O. Thompson, Reg. 36035
Mark Joy, Reg. 35562
Allen E. Hoover, Reg. 37354
David M. Airan, Reg. 38811
Xavier Pillai, Reg. 39799
Y. Kurt Chang, Reg. 41397
Gregory C. Bays, Reg. 40505
Carol Larcher, Reg. 35243
Steven H. Sklar, Reg. 42154
M. Daniel Hefner, Reg. 41826
Thomas A. Belush, Reg. 37090
Kenneth P. Spina, Reg. 43927
Gary R. Jarosik, Reg. 35906
Song Zhu, Reg. 44420
Andrew J. Heinisch, Reg. 43666
Jeffery J. Makeever, Reg. 37390
Salim A. Hasan, Reg. 38175
Richard A. Wulff, Reg. 42238
Jamison E. Lynch, Reg. 41168
Rattan Nath, Reg. 43827
Robert M. Gould, Reg. 43642
Leonard S. Smith, Reg. 43139
Kevin L. Wingate, Reg. 38662
David J. Schodin, Reg. 41294
Paul L. Ahern, Reg. 17020
Theodore W. Anderson, Reg. 17035
Noel I. Smith, Reg. 18698

I further direct that correspondence concerning this application be directed to LEYDIG, VOIT & MAYER, LTD., Two Prudential Plaza, Suite 4900, 180 North Stetson, Chicago, Illinois 60601-6780, Telephone (312) 616-5600.

I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of **sole or first inventor:** Dieter Pelz

Inventor's signature ______________________________

Date ____________________ Country of Citizenship: Austria

Residence: Graz, Austria

Post Office Address: Hans-Mauracher Str. 45, 8044 Graz, Austria

Full name of **second joint inventor,** if any: Gilbert Moser

Inventor's signature _____

Date _____ Country of Citizenship: Austria

Residence: Graz, Austria

Post Office Address: Triesterstrasse 103, 8055 Graz, Austria

Full name of **third joint inventor,** if any: Gerald Zanker

Inventor's signature _____

Date _____ Country of Citizenship: Austria

Residence: Hart bei Graz, Austria

Post Office Address: Schulgasse 1, 8075 Hart bei Graz, Austria

Full name of **fourth joint inventor,** if any Walter Serro

Inventor's signature ______________________________

Date ____________________ Country of Citizenship: Austria

Residence: Ablass, Austria

Post Office Address: 2440 Reisenberg am, Ablass 5, Austria

Full name of **fifth joint inventor,** if any: Volker Ribitsch

Inventor's signature _____

Date _____ Country of Citizenship: Austria

Residence: Graz, Austria

Post Office Address: Heinrichstrasse 28, A-8010 Graz, Austria

Full name of **sixth joint inventor,** if any: Horst Randhahn

Inventor's signature ______________________________

Date ____________________ Country of Citizenship: Germany

Residence: Darmstadt, Germany

Post Office Address: Frankensteiner Str. 58, 642 97 Darmstadt, Germany

Full name of seventh joint inventor, if any: Peter J. Degen

Inventor's signature Peter J Degen

Date 12-22-1999

Residence: Huntington, New York

Country of Citizenship: US

Post Office Address: 24 Glades Way, Huntington, New York 11748